



RAPID COMMUNICATION

Destruction of Neurons in the VPM Thalamus Prevents Rabbit Heart Rate Conditioning

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MCCABE, P. M., M. D. McECHRON, E. J. GREEN AND N. SCHNEIDERMAN. *Destruction of neurons in the VPM thalamus prevents rabbit heart rate conditioning*. *PHYSIOL BEHAV* 57(1) 159–163 1995.—The present study examined the role of the ventral posterior medial nucleus of the thalamus (VPM) in classical heart rate (HR) conditioning using an acoustic conditioned stimulus (CS) and a corneal air puff unconditioned stimulus (US). Previous research suggests that VPM neurons are activated during the presentation of a corneal air puff US. Rabbits were given ibotenic acid lesions in the VPM and subjected to one Pavlovian HR conditioning session. The results of the present study demonstrate that destruction of cell bodies in the VPM reduces HR conditioning to the level of a pseudoconditioning control without affecting HR baseline, or orienting responses to the CS. Lesions of the VPM also significantly augment the tachycardiac unconditioned response, suggesting that VPM lesions alter the somatosensory processing of the US.

Ventral posterior medial nucleus
Rabbit

Somatosensory thalamus

Unconditioned stimulus

Heart rate conditioning

THE present study examined the role of the ventral posterior medial nucleus of the thalamus (VPM) in classical heart rate (HR) conditioning using an acoustic conditioned stimulus (CS) and a corneal air puff unconditioned stimulus (US). Previous studies have shown the medial subnucleus of the medial geniculate (mMG) and the amygdala to be critical sites for acoustic cardiovascular conditioning (7,9,11,16). Both of these sites receive acoustic and somatosensory input (6,10,13,26,27) and exhibit associative changes in neural activity during cardiovascular conditioning (5,19).

Studies have demonstrated that auditory CS information is relayed from the inferior colliculus to the mMG, and from the mMG to the amygdala (2,12). Evidence also suggests that US information produced by an air puff to the cornea is transmitted by the fifth cranial nerve to the trigeminal brain stem complex and the dorsal horn of the spinal cord in the upper cervical segments (15,17,19). Projections from these areas may then serve to transmit US information to the mMG and the amygdala. For example, several studies have shown that the mMG receives projections from the trigeminal brain stem complex (6,9,13,14,20,21).

Although trigeminal nuclei send projections to the mMG, the major trigeminal thalamic projections are to the VPM (3,6,20,24). Neurons in the VPM electrophysiologically respond

to tactile–facial sensation and nociceptive–facial sensation (28,29). Together, this evidence suggests that VPM neurons may be activated during the presentation of a corneal air puff US. However, it is not clear if VPM is part of the pathway that relays US information to the critical sites of sensory convergence in the HR conditioning pathway. Therefore, the present study sought to determine the effects of bilateral VPM lesions on the HR conditioned response (CR), unconditioned response (UR), and orienting response (OR) to the first presentation of the CS. These lesions were produced by injections of ibotenic acid, a substance shown to destroy cell bodies and spare fibers of passage (4).

METHOD

Subjects

Subjects were 26 New Zealand albino rabbits of both sexes (2.0–3.0 kg); however, only 18 animals sustained lesion damage that allowed them to be used in the treatment conditions of the present study. Animals were maintained on a 12-h light/dark cycle with food and water provided ad lib.

Surgery

Animals were anesthetized with gaseous halothane (2.0–3.0% in 100% oxygen) and positioned in a Kopf stereotaxic frame.

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Using aseptic surgical procedures, the scalp was incised, the skull trephined, and the dura punctured. A 1- μ l Hamilton syringe was stereotactically lowered into the thalamus. The brain tissue was allowed to come to equilibrium during a 10-min waiting period before a 500-nl injection of ibotenic acid (7.0 μ g/ μ l in 0.9% saline) was made via the cannula. The injections were made in 50-nl increments every 2 min. Injections were made bilaterally in the VPM or bilaterally in the lateral geniculate nucleus (LG). Following the injection, the cannula was left in place for 15 min to allow the injection vehicle to diffuse from the injection site.

Apparatus

Before conditioning, animals were fitted with electrocardiographic (EKG) recording electrodes and restrained in a sound-attenuating chamber. The EKG signals were amplified using a Grass model 7P5 AC preamplifier. Background white noise (70 dB SPL) and a tone CS (560 Hz; 90 dB SPL; 2-s duration) were provided through an 8 Ω speaker positioned 15 cm anterior to the subject's head. A puff of air delivered to the center of the right cornea served as the US. The air puff US was delivered through a 14-ga blunt hypodermic tube perpendicular to the tangent plane of the globe of the eyeball. A regulator attached to a tank of compressed nitrogen controlled the amount of pressure released by a 28-V Skinner valve in line between the tank and hypodermic tube. The air puff pressure (15 N/cm²) was calibrated at the hypodermic tube using a sphygmomanometer-calibrated Gould linear pressure transducer amplified by a Grass model 7P1 D.C. preamplifier.

Procedure

Prior to behavioral testing, animals were acclimated to the stress of handling and placed in the experimental chamber for 30 min on each of 2 days. On the day of behavioral testing, animals were placed in the experimental chamber and given habituation trials [intertrial interval (ITI) = 90 ± 10 s] in which the CS was presented without the US. The CS was presented randomly for 18–54 trials (until the bradycardiac response to the CS was no more than -7.5 bpm). The first habituation trial was used to examine the HR OR to the tone CS.

Immediately after habituation, animals with lesions in the VPM ($n = 6$) and control lesions in the LG ($n = 6$) received one Pavlovian conditioning session (30 trials; ITI = 90 ± 10 s). The conditioning session consisted of 26 trials in which the CS was paired with the US and four trials that served as test trials. During CS–US pairings the US onset occurred simultaneously with the offset of the CS. Trials 7, 15, 22, and 30 served as the test trials on which the CS was presented alone (CS test trials). Following the last CS test trial, each animal also received five US test trials in which the US was presented alone.

To examine the nonassociative HR responses to the stimuli, an additional group of animals with bilateral LG lesions ($n = 6$) was given a pseudoconditioning session. The pseudoconditioning session was administered immediately after the habituation session. Pseudoconditioning consisted of 60 presentations of the CS and US in an explicitly unpaired fashion (ITI = 45 ± 5 s). Trials 15, 30, 45, and 60 served as CS test trials. The remaining 28 CS-alone trials and 28 US-alone trials were presented pseudorandomly with the limitation that the same stimulus was presented on no more than two consecutive trials. Following the last CS test trial of pseudoconditioning, each animal also received five US test trials in which the US was presented alone. Total session time and the number of tones and shocks was the same for both the conditioning and pseudoconditioning procedures.

Histology

At the end of the conditioning and pseudoconditioning sessions, animals were overdosed with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 4% formalin. The brain was removed and soaked in a formalin-sucrose solution for several days before sectioning. Alternate frozen coronal sections (40 μ m) through the lesioned area were mounted on gelatin-coated slides and stained with Cresyl violet for Nissl preparation. Sections were traced and the lesions plotted under a magnification of 10 $\times$ using a Nikon microscope and drawing tube. Drawings were then used to estimate the extent of damage caused by the lesion. A magnification of 40 $\times$ was used to estimate areas of visible necrosis surrounding the lesion. All areas of necrosis were included in damage estimates.

Analyses

The HR was computed by measuring successive five-beat R–R intervals read to the nearest 0.5 mm from the polygraph chart at a 25 mm/s paper speed. For the purposes of subsequent analyses, these five beat R–R intervals will be referred to as intervals. On each test trial, the interval immediately preceding stimulus onset was taken as the prestimulus baseline.

Measures of HR were computed on OR, CS, and US test trials. The HR OR for each animal was computed as the largest bradycardiac response of the three consecutive intervals following the prestimulus baseline interval on the first habituation trial. On CS test trials the three consecutive intervals following the prestimulus baseline interval were measured to examine the HR CR. On US test trials the five intervals following the prestimulus baseline interval were measured to examine the HR UR.

Analyses of variance (ANOVAs) were conducted on mean baseline HR, HR ORs, HR CRs, and HR URs. Each analysis included a group factor that divided animals into three conditions: animals with lesions of VPM that received conditioning (VPM group); animals with lesions of LG that received conditioning (control lesion group); and animals with lesions of LG that received pseudoconditioning (pseudo group). A mean baseline HR was computed for each animal by averaging the prestimulus baseline measures from each test trial. A preliminary analysis of mean baseline HR was conducted using a one-way ANOVA that compared the three groups. The preliminary analysis revealed no difference in baseline HR between groups. Therefore, all data for subsequent analyses were expressed as change scores from prestimulus baseline measures. Change scores were computed for the HR CRs and HR URs by calculating the average HR of each pre- and poststimulus interval across the respective test trials, and subtracting the prestimulus interval average from each of the poststimulus interval averages (intervals). The HR CRs were analyzed using a factorial ANOVA that included a 3 group $\times$ 3 intervals repeated-measures design. The HR URs were analyzed using a factorial ANOVA that included a 3 group $\times$ 5 intervals repeated-measures design. The HR OR of the three treatments was analyzed using a one-way ANOVA. Post hoc Newman–Keuls stepwise group comparisons were used to test mean differences. An alpha level of 0.05 was required for statistical significance in ANOVAs and post hoc tests.

RESULTS

Histology

Figure 1 shows one coronal section from each of the animals that received Pavlovian conditioning and were found to have bilateral damage in VPM (VPM group; cases V1–V6). Animals

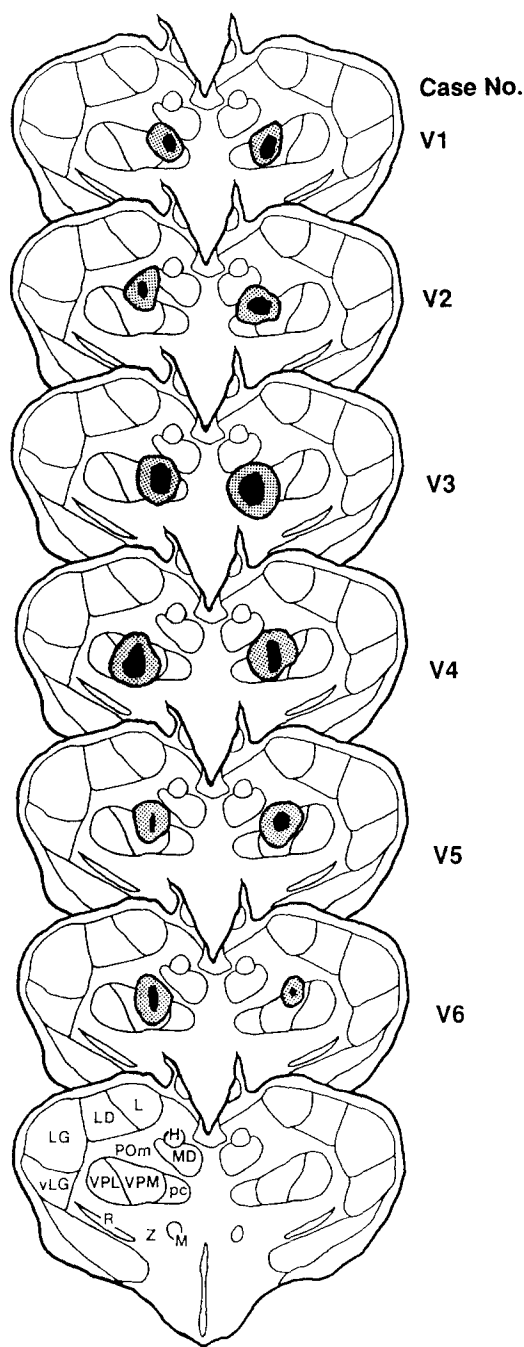


FIG. 1. One coronal section is shown from each of the animals (cases V1 through V6) that received Pavlovian conditioning and were found to have bilateral damage to the ventral posterior medial nucleus of the thalamus. The blackened area represents the injection site and the shaded area represents the necrotic damage. Unlike control animals, this group exhibited augmented tachycardiac unconditioned responses and heart rate conditioning that were not different from the pseudoconditioning group. H, habenulae nucleus; L, lateral thalamus; LD, lateral dorsal nucleus of the thalamus; LG, lateral geniculate nucleus; M, mammillary tract; MD, medial dorsal nucleus of the thalamus; pc, parvocellular region of the ventral posterior medial nucleus of the thalamus; POM, medial portion of the posterior complex of the thalamus; R, reticular nucleus of the thalamus; vLG, ventral portion of the lateral geniculate nucleus; VPL, ventral posterior lateral nucleus of the thalamus; VPM, ventral posterior medial nucleus of the thalamus; Z, zona incerta.

were placed in the VPM group if each unilateral VPM sustained greater than 25% damage. Damage to the area just dorsal to VPM, the medial portion of the posterior complex (POM), was unavoidable. However, great care was taken to select only those animals that received the least amount of damage to POM. Similarly, animals that received significant damage to the medial dorsal nucleus of the thalamus (MD) were not included in the VPM group.

All VPM lesions were placed approximately at the rostro-caudal level of the sections shown in Fig. 1. Damage to the VPM was bilateral and extensive (>50%) in cases V3, V4, and V5, and bilateral and significant (>25%) in cases V1, V2, and V6. Damage to the parvocellular region of VPM was: bilateral and extensive (>30%) in cases V1 and V3; bilateral and minimal (<10%) in case V5; and unilateral and significant (20%) in cases V2, V4, and V6. Damage to the POM was bilateral and significant (>30%) in each case. Damage to the MD was unilateral and minimal (<5%) in case V1, and bilateral and minimal (<10%) in cases V2 and V3. Damage to the ventral posterior lateral nucleus of the thalamus was bilateral and minimal (<20%) in case V4.

All control animals that received Pavlovian conditioning (control lesion group; $n = 6$) and pseudoconditioning (pseudo group; $n = 6$) had lesions centered in the LG. Two of the animals with LG lesions also received necrotic damage along the dorsal border of ventral LG.

Orienting Response

The HR OR consisted of bradycardia for all animals except one animal in the pseudo group. The HR ORs for the VPM, control lesion, and pseudo groups were -36.8 (SE = 9.2), -41.7 (SE = 5.5), and -26.6 (SE = 6.2), respectively. There was no significant difference in HR ORs among the three groups, $F(2, 15) = 1.16$, $p = 0.339$.

Conditioned Response

The upper panel of Fig. 2 shows the HR CRs across three intervals for the VPM and control lesion groups with respect to the pseudo group. The HR CR for each of the groups was a bradycardiac response that was greatest in magnitude at the second interval. An analysis revealed significant main effects of intervals and groups [$F(2, 30) = 4.63$, $p = 0.018$, and $F(2, 15) = 8.46$, $p = 0.003$, respectively]. Post hoc tests of mean differences were conducted on the intervals main effect ($MS_{\text{error}} = 8.59$). The post hoc tests revealed that the HR CR at interval 2 was significantly greater than at intervals 1 and 3. Post hoc tests of mean differences were also conducted on the groups main effect ($MS_{\text{error}} = 158.83$). The post hoc tests revealed that the HR CR for the control lesion group was significantly greater than the HR CR for the VPM and pseudo groups. There was no significant difference in HR CRs between the VPM and pseudo groups. These findings indicate that the destruction of cell bodies in the VPM reduces HR CRs to the nonassociative level of HR pseudoconditioning.

Unconditioned Response

The average HR UR to the air puff alone is shown in the lower panel of Fig. 2 for the VPM, control lesion, and pseudo groups across the five intervals. The HR UR for the VPM group was a large tachycardiac increase from baseline across each interval, maximal at intervals 2 and 3. The HR UR for the control lesion group was an initial bradycardia at interval 1 and small tachycardia at intervals 2 and 3, followed by bradycardia at intervals

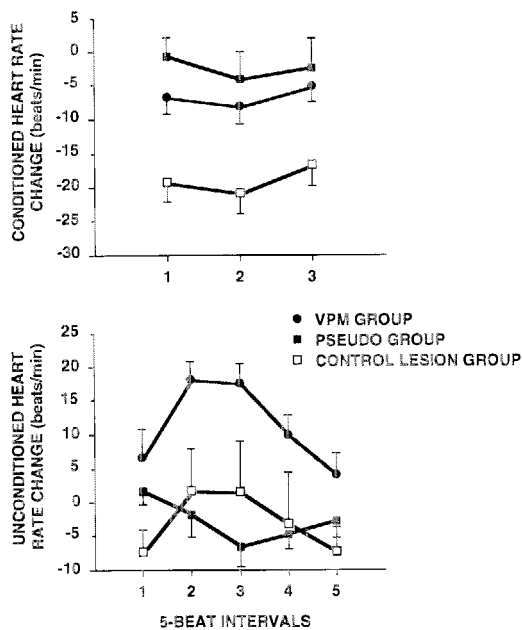


FIG. 2. The mean heart rate (HR) conditioned responses (upper panel) and unconditioned responses (lower panel). The HR is expressed as a change from prestimulus baseline (in bpm) across five-beat R-R intervals. Bars indicate SEM. Animals with control lesions (control lesion group; open square; $n = 6$) showed greater HR conditioning than animals with VPM lesions (VPM group; shaded circle; $n = 6$) and animals that received pseudoconditioning (pseudo group; black square; $n = 6$). The VPM group showed an augmented tachycardiac unconditioned response compared to the pseudo and control lesion groups.

4 and 5. The pseudo group showed an initial small tachycardia at interval 1 followed by bradycardia. An analysis revealed significant main effects of intervals and groups [$F(4, 60) = 2.65$, $p = 0.042$, and $F(2, 15) = 9.47$, $p = 0.002$, respectively]. Post hoc tests of mean differences were conducted on the intervals main effect ($MS_{\text{error}} = 68.23$). The post hoc tests revealed that the HR UR at interval 2 was significantly greater than at interval 5. Post hoc tests of mean differences were also conducted on the groups main effect using a $MS_{\text{error}} = 207.67$. The post hoc tests revealed that the HR UR for the VPM group was significantly greater than the HR UR for the control lesion and pseudo groups. There was no significant difference in HR URs between the control lesion and pseudo groups. These findings indicate that the destruction of cell bodies in VPM significantly augments the tachycardiac UR.

DISCUSSION

The present study demonstrated that the destruction of cell bodies in the VPM reduces HR conditioning to the level of pseudoconditioning without affecting HR baseline or ORs. These findings provide evidence that the VPM is a critical site in the

HR conditioning pathway. Furthermore, lesions of the VPM significantly augmented the tachycardiac UR, suggesting that lesions of the VPM prevent HR conditioning by altering the somatosensory processing of the corneal air puff US.

This interpretation is supported by previous studies showing that the VPM is involved in the processing of facial somatosensory information (28,29), and the sensory discriminative aspects of pain (23). Thus, VPM lesions in the present study may have prevented HR conditioning by disrupting the transmission of US information to other sites in the HR conditioning pathway that receive CS and US inputs. For example, the VPM may be part of a circuit that relays US information to the mMG and/or the amygdala. The mMG and the amygdala are both potential sites of plasticity (5,19) in the HR conditioning circuit that receive acoustic and somatosensory inputs (6,10,13,26,27).

It is interesting that VPM lesions did not abolish the HR UR, but produced an augmentation of the tachycardiac UR. It is possible that the VPM lesions disrupted US input to critical areas necessary for HR conditioning, and at the same time disinhibited a portion of the circuit that is involved in the production of HR URs. This seems likely considering evidence that neurons in the VPM have inhibitory connections with other thalamic neurons (25). On the other hand, these results do not rule out the possibility that VPM lesions altered the HR UR independent of the transmission of US information to other critical areas of HR conditioning. This seems unlikely considering the role of the VPM in facial sensation (23,28,29). Nevertheless, these results clearly show that the VPM is a critical site in the HR conditioning pathway.

The MD has been shown to be an important thalamic structure involved in the late phase tachycardiac component of HR conditioning that is believed to reflect sympathetic responding (1). However, it is unlikely that the results of the present study were due to MD damage because: 1) VPM lesions prevented early bradycardiac CRs; and 2) only three of the animals in the VPM group sustained minimal ($<10\%$) damage to the MD. On the other hand, it is possible that the animals in the VPM group would have shown some other form of HR conditioning, such as sympathetic responding, if longer CSs or longer training sessions were used.

Some researchers have suggested that the POM is critical for the sensation of noxious stimuli (22). The present study sought to minimize damage to the POM; however, each of the animals in the VPM group sustained some necrotic damage (up to 30%) to the POM. Thus, we do not discount the possibility that the limited damage to the POM may have played a role in the prevention of HR conditioning and/or the augmentation of the tachycardiac UR.

In summary, the destruction of cell bodies in VPM prevents Pavlovian HR conditioning, and also significantly augments the tachycardiac UR. These results suggest that VPM lesions prevent HR conditioning by altering the somatosensory processing of the corneal air puff US.

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